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CURRENT PERSPECTIVE ESSAY

Redox Homeostasis and Antioxidant Signaling: A Metabolic Interface between Stress Perception and Physiological Responses

Low molecular weight antioxidants, such as ascorbate, glutathione, and tocopherol, are information-rich redox buffers that interact with numerous cellular components. In addition to crucial roles in defense and as enzyme cofactors, cellular antioxidants influence plant growth and development by modulating processes from mitosis and cell elongation to senescence and death (De Pinto and De Gara, 2004; Potters et al., 2004; Tokunaga et al., 2005). Most importantly, antioxidants provide essential information on cellular redox state, and they influence gene expression associated with biotic and abiotic stress responses to maximize defense. Growing evidence suggests a model for redox homeostasis in which the reactive oxygen species (ROS)–antioxidant interaction acts as a metabolic interface for signals derived from metabolism and from the environment. This interface modulates the appropriate induction of acclimation processes or, alternatively, execution of cell death programs.

THE CONCEPT OF REDOX HOMEOSTASIS IN PLANTS

Efficient flux through plant electron transport cascades requires the simultaneous presence of both oxidized and reduced forms of electron carriers. This requirement, known as redox poising, involves a continuous flux of electrons to molecular oxygen from multiple sites in the photosynthetic and respiratory electron transport chains. Apart from the specialized water-producing reactions catalyzed by specific oxidases, the initial product of this flux is superoxide, from which other ROS are subsequently produced (Table 1). Singlet oxygen is also formed during light capture and photochemistry. Numerous enzyme systems produce superoxide or H_2O_2. The reactive nature of these intermediates means not only that their accumulation must be controlled but also that they are able to act as signaling molecules.

The extent to which ROS accumulate is determined by the antioxidative system, which enables organisms to maintain proteins and other cellular components in an active state for metabolism (Figure 1). Like all other aerobic organisms, plants maintain most cytoplasmic thiols in the reduced (−SH) state because of the low thiol-disulfide redox potential imposed by millimolar amounts of the thiol buffer, glutathione. Unlike many animal cells, however, plant cells synthesize high concentrations of ascorbate (vitamin C), an additional hydrophilic redox buffer that provides robust protection against oxidative challenge. Redox homeostasis is governed by the presence of large pools of these antioxidants that absorb and buffer reductants and oxidants (Figure 1). Plants also make tocopherols (vitamin E) that act as important liposoluble redox buffers. Although tocopherol is considered to be a major singlet oxygen scavenger, it is also an effective scavenger of other ROS, and in this case the reduced scavenging form may be regenerated by ascorbate (Foyer et al., 2005). Moreover, because the tocopherol redox couple has a more positive midpoint potential than that of the ascorbate pool, it increases even further the range of effective superoxide scavenging. The ability of the ascorbate, glutathione, and tocopherol pools to act as redox buffers in plant cells is one of their most important attributes.

Pathways of ROS signaling are made possible by homeostatic regulation achieved by antioxidant redox buffering. Because antioxidants continuously process ROS, they determine the lifetime and the specificity of the ROS signal. Plant cells generally cope very well with high rates of generation of superoxide, H_2O_2, and even singlet oxygen. Although cellular oxidation is important in all abiotic and biotic stress responses, the extent and physiological significance of oxidative damage is debatable. For example, plants with low activities of both catalase and cytosolic ascorbate peroxidase show less severe stress symptoms than plants that lack either one of these enzymes (Rizhsky et al., 2002). It now appears that singlet oxygen-mediated cell death is not a direct result of damage per se but rather is genetically programmed via the EXECUTOR1 pathway (Wagner et al., 2004). Moreover, plants adapt very well to depletion of antioxidants by signaled induction of other defense systems. For example, tocopherol-deficient Arabidopsis *vte* mutant seedlings have high amounts of lipid peroxides, but the mature plants display only a slightly abnormal phenotype (Kanwischer et al., 2005). One presumes that rapid protein turnover or DNA repair is increased to compensate for increased oxidation or loss of antioxidants in these circumstances.

REACTIVE OXYGEN-ANTIOXIDANT INTERACTIONS IN REDOX SIGNALING

The last 5 years have seen a radical change in the appreciation of the pivotal importance of antioxidant status. In addition to its roles in removing ROS, antioxidant status appears to set the threshold for general plant defense responses, particularly those provoked by biotic stresses and wounding. Indeed, modulation of the ROS-antioxidant interaction plays a part in many stresses, as well as other responses to the environment, and in the regulation of plant development. Symbiotic associations between organisms also involve ROS-antioxidant interactions, and this can lead to an enhancement of antioxidant status such that the

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Table 1. Approximate Redox Potentials and Intracellular Concentrations of Major Redox Couples and Rate Constants for Reaction of Ascorbate and Glutathione with ROS[a]

Redox Couple[c]	Redox Potential (V)	Concentration Range (μM)[d]	Rate Constants for Reactive Oxygen Scavenging[b]	
			Singlet Oxygen[e]	Superoxide[f]
O_2/H_2O	+0.82	200–300 (O_2)		
$O_2/O_2^{\cdot -}$	−0.30	<0.001 ($O_2^{\cdot -}$)		
$O_2^{\cdot -}/H_2O_2$	+0.94	1–100 (H_2O_2)		
$H_2O_2/OH^{\cdot}$	+0.54	Negligible ($OH^{\cdot}$)[g]		
$OH^{\cdot}/H_2O$	+2.20	–		
DHA/ASC	−0.10	10,000–20,000	1×10^7	2×10^5
GSSG/GSH	−0.24	2,000–5,000	2×10^6	7×10^5
TRX_{ox}/TRX_{red}	−0.33	10–100		
NAD(P)/NAD(P)H	−0.32	200–500		
Fd_{ox}/Fd_{red}	−0.42	10–100		

[a] References: Asada and Takahashi (1987), Bors et al. (1989, 1990), and Polle (2001).
[b] The rate of uncatalyzed reaction with ROS depends on concentration and reaction rate constant, here given in $M^{-1}s^{-1}$.
[c] ASC, ascorbate; DHA, dehydroascorbate; Fd_{ox}, oxidized ferredoxin; Fd_{red}, reduced ferredoxin; GSH, glutathione; GSSG, glutathione disulfide; TRX_{ox}, oxidized thioredoxin; TRX_{red}, reduced thioredoxin.
[d] High intracellular concentrations of antioxidants prevent indiscriminate oxidation of key cellular components by maintaining oxidant concentrations low. In addition to the abundant pools of ascorbate and glutathione and the battery of peroxide processing enzymes, numerous other compounds can act as chemical antioxidants, including tocopherols, quinones, lipoic acid, carotenoids, and flavonoids.
[e] Rate constants of membrane-associated antioxidants for reaction with singlet oxygen are approximately 1×10^{10} (carotenoids) and 3×10^8 (α-tocopherol).
[f] Rate constants of some phenolic compounds, flavonoids, and polyamines for reaction with superoxide are of the order of 5×10^5 (kaempferol), 4×10^5 (caffeic acid), 9×10^4 (quercetin), 3×10^2 (spermidine), and 1×10^2 (putrescine).
[g] The hydroxyl radical and singlet oxygen are so reactive that their concentrations are considered to be negligible.

symbiotic partnership is more resistant to environmental stress than either partner alone (Kranner et al., 2005). Any stimulus that perturbs cellular redox balance may serve as an inducer for the same set of defense-related genes, including pathogenesis-related (PR) proteins. Thus, low levels of ascorbate, for example, can act as an elicitor of pathogen resistance responses (Pastori et al., 2003; Barth et al., 2004), as can changes to the cellular glutathione pool (Mou et al., 2003; Gomez et al., 2004).

Most attention has focused on the apoplast as a site where oxidants are produced and perceived. A key point, therefore, concerns the antioxidant status of the apoplast. Despite the presence of many antioxidants, such as flavonoids and polyamines, the redox buffering capacity of the apoplast is much weaker than inside the cell (Horemans et al., 2000; Pignocchi et al., 2003). Unlike the cytoplasm, the apoplast is deficient in NAD(P)H and glutathione and contains a very active and regulated ascorbate oxidase. Thus, apoplastic ascorbate is markedly more oxidized than cytoplasmic ascorbate (Pignocchi et al., 2003). Moreover, the pathway for ascorbate degradation has recently been shown to be located in the apoplast (Green and Fry, 2005).

Membrane ascorbate and dehydroascorbate transport systems fail to maintain a highly reduced apoplastic ascorbate pool. Nonetheless, an ascorbate-based system could be important in driving plasma membrane and tonoplast electron transport chains. Both membranes contain an ascorbate-dependent cytochrome *b*561, which is reduced on one face by ascorbate and oxidized on the other by monodehydroascorbate or phenolics that can act as substrates for monodehydroascorbate reductase (Preger et al., 2004).

The ultimate electron acceptor in the apoplast is either oxygen or 3,4-dihydroxyphenolic compounds such as chlorogenic acid, caffeic acid, quercetin, and catechin that influence cell wall composition. These reactions, acting together with ascorbate oxidase, are part of a futile cycle that regulates production of both reduced ascorbate and oxidized forms (monodehydroscorbate and dehydroascorbate) in the apoplastic environment for functions in metabolism and growth. Such a cycle may be crucial in controlling cell expansion and in facilitating ROS-mediated signal transmission that occurs in response to atmospheric pollutants, pathogens, or hormones (Pignocchi et al., 2003). It is intriguing that another, as yet uncharacterized, apoplastic protein, encoded by *VTC2*, is involved in ascorbate accumulation in leaves.

Our current knowledge of redox controls in the apoplast and cytoplasm predicts that (1) the plasma membrane is an important site for perception and transduction of environmental change through redox signals; (2) apoplastic redox changes facilitate interactions between receptor proteins containing oxidizable thiols that are sited in or near the membrane surface; (3) because the apoplastic redox buffering capacity is low, a steep redox gradient is present across the plasma membrane; (4) this gradient triggers or elaborates membrane channel activity involving calcium release and aquaporin or peroxiporin function. Moreover, low apoplastic antioxidant buffering permits further reactions to be

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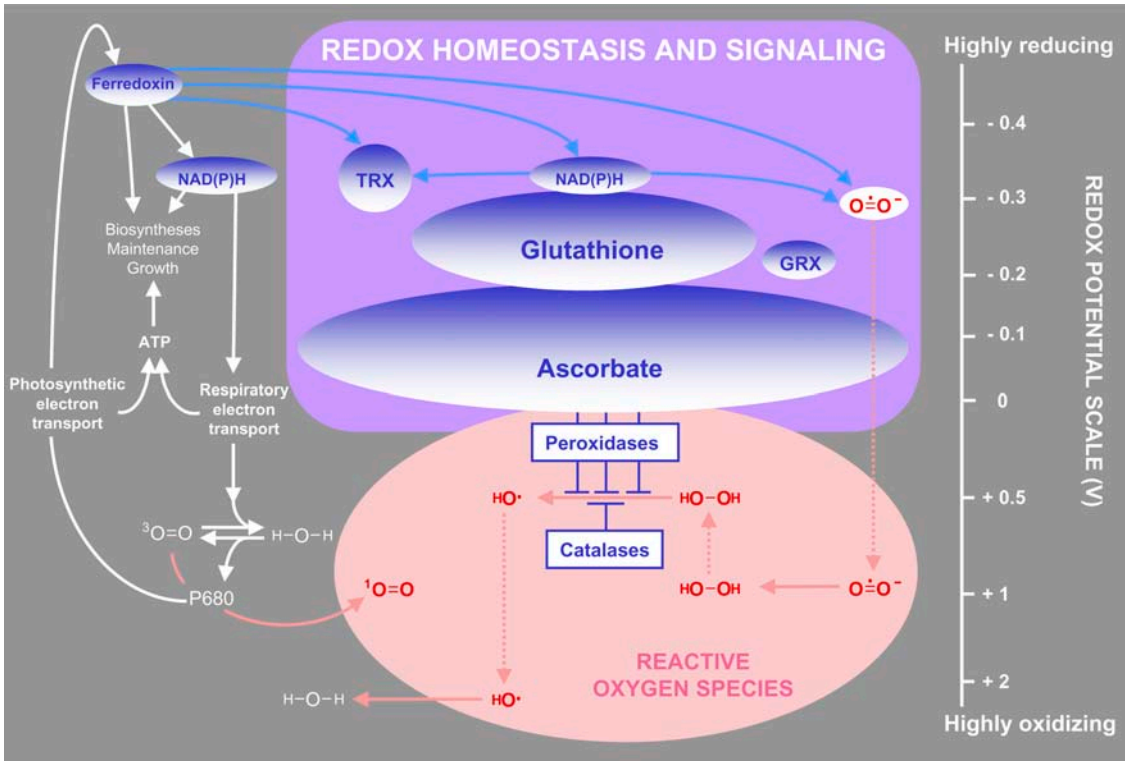

Figure 1. Reductant–Antioxidant–Oxidant Interactions in Redox Homeostasis and Signaling.

Nonenzymic components are positioned on a nonlinear scale (right) at their approximate electrochemical potential in volts. In nonquiescent cells under optimal conditions, large pools of glutathione and ascorbate are maintained in a highly reduced state, and buffer ROS that are continuously produced by oxidases or by electron transport components, such as FeS centers, semiquinones, or (as depicted) ferredoxin. Other key redox signaling components are thioredoxins (TRX) and glutaredoxins (GRX), which are reduced by ferredoxin, NADPH, or glutathione. Production of the superoxide anion ($OO^{\cdot -}$) and H_2O_2 (HOOH) can be induced or promoted under certain conditions, leading to increased oxidative charge on the reductant-antioxidant system. Reductive cleavage of H_2O_2 produces the hydroxyl radical ($OH^{\cdot}$), an extremely reactive electron and hydrogen acceptor whose reduction potentially involves indiscriminate oxidation of cellular components. Excessive production of $OH^{\cdot}$ is avoided by enzymatic processing of H_2O_2 to water by peroxidases or to water and O_2 by catalases. Signaling linked to increased availability of ROS may be caused, limited, or mediated by changes in antioxidant capacity (see text).

triggered by secondary oxidant-induced signaling events in the cell wall, such as release of small oligosaccharides generated during the breakdown of pectins.

In addition to the apoplast, the thylakoid lumen is another compartment in which low antioxidant buffering may heighten or facilitate redox signaling. The thylakoid membrane transports ascorbate by diffusion alone (Foyer and Lelandais, 1996; Horemans et al., 2000), despite the location of key ascorbate-requiring reactions such as violaxanthin de-epoxidation on the internal face of the membrane. Low antioxidant buffering could allow oxidative signals to accumulate within the lumen, and this could be important in redox signal transduction leading to programmed cell death (PCD).

ANTIOXIDANTS AND REDOX SENSING MECHANISMS

Signaling mediated by ROS involves heterotrimeric G-proteins (Joo et al., 2005) and protein phosphorylation regulated by specific MAP kinases and protein Tyr phosphatases (Kovtun et al., 2000; Gupta and Luan, 2003; Rentel et al., 2004). The biochemical and structural basis of kinase pathway activation by ROS remains to be established in plants, but thiol oxidation likely plays a key role. The best-characterized redox signal trandsuction system in plants is the stromal ferredoxin-thioredoxin system, which functions in the regulation of photosynthetic carbon metabolism. Signal transmission involves disulfide-thiol conversion in target enzymes and is probably achieved by a light-induced decrease in the thioredoxin redox potential from approximately −0.26 V (dark: 20% reduced thioredoxin, pH 7) to approximately −0.36 V (light: 90% reduced thioredoxin, pH 8) (Setterdahl et al., 2003). Thiol groups are likely important in other types of redox signal transduction, including ROS sensing by receptor kinases, such as ETR1 (Desikan

et al., 2005). Thiol-based regulation may be important in plant acclimation to environmental change, particularly where redox interactions play a key role in the orchestration of the abiotic stress response. The heat shock response, for example, can be completely inhibited by effective removal of H_2O_2 because the expression of genes such as those encoding HSF21 and HSF5 and cytosolic APX1 is modulated by ROS signals (Davletova et al., 2005).

In plants, as in other organisms, thiol-containing domains are oxidized by ROS, either directly or indirectly, to give relatively stable oxidation products with modified physical conformations or biochemical activities. Both the bacterial oxyR and the yeast yAP-1 transcription factors are activated through protein thiol oxidation by peroxides (Bauer et al., 1999; Delauney et al., 2002). Similarly, mammalian heat shock factor 1, which is a key player in the response to H_2O_2 and other stresses, is activated by oligomerization driven by oxidant-induced disulfide bond formation (Ahn and Thiele, 2003). In addition to disulfides, other oxidized species of Cys sulfur that may be important in redox sensing include sulfenic acid and glutathionylated Cys (Figure 2), sulfenyl amide groups, and sulfur-metal bonds. These signaling mechanisms involve relatively direct effects of ROS on proteins, particularly transcription factors, which result in changes in gene expression.

Thermodynamics (redox potential of oxidizable thiols) and kinetics (ability to compete with the antioxidative system) are key considerations in assessing the functional importance of putative thiol-based ROS sensors in plants. The H_2O_2-reactive thiols in oxyR have a midpoint redox potential of approximately −0.18 V and a rate constant for reaction with H_2O_2 that is comparable to peroxidases. These properties mean that the protein generally will be in its reduced, inactive form under optimal conditions, when the redox potential of the glutathione pool is approximately −0.24 V, but that increases in H_2O_2 availability or changes in glutathione redox potential (or both) can readily cause oxidative activation of the sensor. Because of the low redox buffering in the apoplast, sensors located in this compartment may have different properties compared with those operating intracellularly. Sensor oxidation may also be facilitated by programmed withdrawal of the antioxidant system or could be catalyzed by specific peroxidases, as shown for the yAP-1 system in yeast (Delauney et al., 2002).

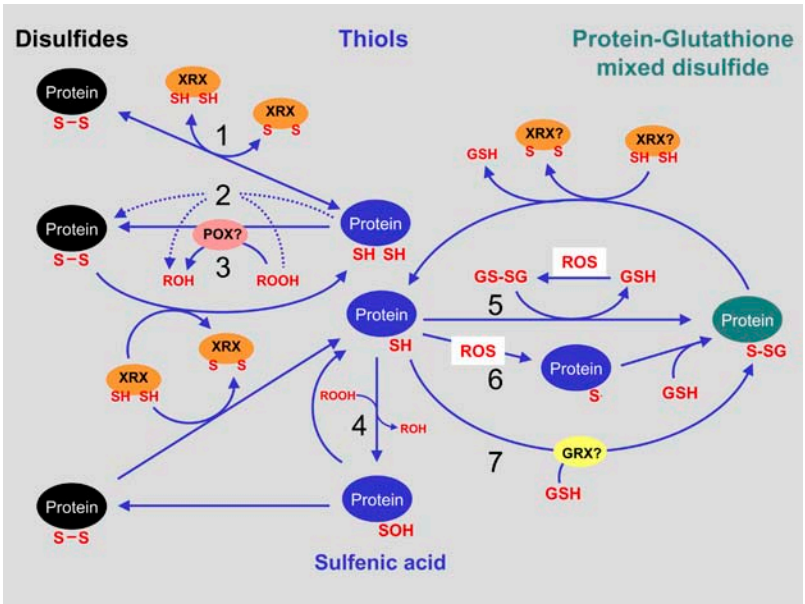

Figure 2. Sensitive Control of Cellular Redox Responses by Protein Thiol Status.

The numbered reactions are as follows. 1, Reversible dithiol-disulfide formation mediated by the thioredoxin (or glutaredoxin) system, as in light modulation of chloroplastic enzyme activities. 2, Direct H_2O_2-dependent oxidation of dithiol sensor proteins, such as the bacterial oxyR, reversed by GRX. 3, Peroxidase-catalyzed H_2O_2 sensing, as occurs with the yeast yAP-1 protein, which can be reversed by TRX. 4, Sulfenic acid formation, as occurs in the bacterial transcription factor OhrR or in peroxiredoxins. The sulfenic acid can be directly reduced by glutathione or lipoic acid (in 1-Cys peroxiredoxins) or give rise to an intramolecular or intermolecular disulfide bond that is reducible to thiols by thioredoxins or glutaredoxins in 2-Cys peroxiredoxins. 5, Glutathionylation through thiol-disulfide exchange of protein thiols with GSSG. 6, ROS-catalyzed glutathionylation via thiyl radical formation. 7, Glutaredoxin-catalyzed glutathionylation reaction. Whatever the route of glutathionylation, the process may be reversed by certain glutaredoxins and/or thioredoxins. Only some of the possible routes for changes in Cys sulfur status are shown. Other modifications, such as protein thiol nitrosylation, could also be important, as could more oxidized forms of protein Cys sulfur (sulfinic and sulfonic acids) and glutathionylation via nitrosylated glutathione. GRX, glutaredoxin; GSH, glutathione; GSSG, glutathione disulfide; POX, peroxidase; TRX, thioredoxin; XRX, thioredoxin or glutaredoxin.

Whereas redox adjustments are central to most stress responses, the extent to which intracellular ROS concentrations increase as a result of stress is highly variable. Many observations suggest that changes in glutathione status may be as important as enhanced ROS pools in redox signaling (Creissen et al., 1999; Vanacker et al., 2000; Mou et al., 2003; Ball et al., 2004; Gomez et al., 2004; Evans et al., 2005). The glutathione pool is oxidized and enhanced in catalase-deficient plants (Noctor et al., 2002; Rizhsky et al., 2002) and in incompatible plant–pathogen interactions (Vanacker et al., 2000). Glutathione is essential for the turnover of the cell cycle and root and nodule meristem activity. The Arabidopsis *rml1* mutant, in which roots fail to develop, was shown to be glutathione deficient because of low activity of γ-glutamylcysteine synthetase (Vernoux et al., 2000), a key enzyme in

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glutathione synthesis that has recently been shown to be located in plastids (Wachter et al., 2005). Moreover, accumulation of GSSG is often associated with tissue death or quiescence.

A key question concerns how glutathione status might be perceived by the cell. Although several mechanisms are possible, current knowledge suggests a potentially important role for protein glutathionylation (i.e., the formation of a mixed disulfide bond between glutathione and specific Cysteine residues) (Figure 2). This posttranslational modification can modulate enzyme activity by modification of catalytic site Cys residues or affect biological activity by competing with other thiol modifications. Increased GSSG may be sufficient to trigger protein glutathionylation, although ROS-catalyzed generation of protein thiyl radicals may allow glutathionylation in the absence of GSSG accumulation. Glutathionylation may also occur independently of enhanced ROS production or redox perturbation of the glutathione pool through induction or activation of enzymes that are able to catalyze the reaction, such as glutaredoxins (Figure 2). In plants, so far, two Calvin cycle enzymes (aldolase and triose phosphate isomerase) have been found to be targets for glutathionylation (Ito et al., 2003). In some cases, glutathionylation could act as a protective mechanism by preventing formation of sulfenic acid groups (Figure 2). Other roles could include buffering the cellular redox potential by preventing excessive accumulation of free GSSG and signal reinforcement during ROS sensing. One important function of glutathionylation in ROS signaling in animals could be through its antagonistic effects on distinct MAP kinase activities (Cross and Templeton, 2004). Further work is required to elucidate the importance of protein glutathionylation in redox signaling processes operating in plants.

In bacteria and yeast, oxidative activation of ROS sensors is reversed by glutaredoxin or thioredoxin (Figure 2). In plants, thioredoxins and glutaredoxins are encoded by multigene families, and targeted proteomic studies are extending the list of proteins that are potential thioredoxin targets in photosynthetic organisms (Balmer et al., 2004; Lemaire et al., 2004; Yamazaki et al., 2004). As well as their established roles in the regulation of the enzymes of carbon metabolism, thioredoxins may act to modulate the activity of key antioxidative enzymes, such as ascorbate peroxidase and catalase (Lemaire et al., 2004; Yamazaki et al., 2004). Such responses might be crucial to redox signal transduction in light- and pathogen-triggered PCD responses (Mateo et al., 2004). Certain glutaredoxins and thioredoxins play direct roles in the antioxidative system by regenerating peroxiredoxins oxidized by peroxides (Rouhier et al., 2001; Collin et al., 2004; Rey et al., 2005). In addition to their antioxidant function, peroxiredoxins could play roles in redox signaling through oxidant-induced oligomerization (König et al., 2002) or perhaps as catalysts of ROS sensor oxidation, a reaction that might be analogous to oxidation of yAP-1 by the thiol-based peroxidase *Gpx3* (Orp1; Delauney et al., 2002).

Recent data suggest that ROS-triggered increases in cytosolic calcium could also be influenced by glutathione status (Gomez et al., 2004; Evans et al., 2005). There is an intimate connection between ROS production and intracellular increases in calcium, which are both early events during pathogen responses (Dangl and Jones, 2001; Lecourieux et al., 2002). Increases in cytosolic calcium are also triggered by ROS production during root hair formation and abscisic acid signaling (Foreman et al., 2003; Kwak et al., 2003).

ANTIOXIDANTS AND STRESS-INDUCED PCD

A current hypothesis is that all cells survive by suppression of a default death pathway that is a legacy from the host–endosymbiont interactions through which eukaryotic cells evolved (Ameisen, 2002). The default death pathway, PCD, is an integral part of many development and defense processes and can be triggered by ROS and reactive nitrogen species. The best-studied PCD process in plants is the hypersensitive response to incompatible pathogens (Dangl and Jones, 2001). Apoplastic ROS accumulation during the hypersensitive response follows activation or induction of ROS generating systems.

The pathogen-induced apoplastic oxidative burst alone may be insufficient to trigger PCD (Mur et al., 2005). However, ROS accumulation in organelles such as mitochondria may also play a role during PCD (Maxwell et al., 2002). Genetic alteration of the mitochondrial electron transport chain desensitizes the plant to stress-induced cell death (Dutilleul et al., 2003). Chloroplasts were shown to be the site of the earliest accumulation of ROS in the guard cells of ozone-fumigated Arabidopsis leaves, with apoplastic ROS accumulation occurring only later as a result of activation of the *AtrbohD-* and *AtrbohF*-encoded NADPH oxidases (Joo et al., 2005). The chloroplast response was extremely rapid and involved the participation of intracellular G-proteins and associated signaling pathways that participate in the stress-elicited oxidative burst (Joo et al., 2005). Other evidence also points to an important role for H_2O_2 generated during photosynthesis in chloroplasts and through photorespiration in PCD (Mateo et al., 2004).

Among numerous lesion mimic mutants that more readily undergo PCD than wild-type plants, *lsd1* has been particularly useful in elucidating the role of superoxide (Jabs et al., 1996) and H_2O_2 (Mateo et al., 2004). It is possible that LSD1 binds to the C-terminal region of the transcription factor AtbZIP10, blocking its entry to the nucleus and retaining it in the cytosol. Such basic domain/leucine zipper transcription factors play key roles in the orchestration of many plant responses to the environment, particularly light and stress signaling. The absence of the LSD1 protein function results in the initiation and propagation of PCD under growth conditions that are optimal for wild-type Arabidopsis, such that exposure to even low light causes lesion formation on the *lsd1* leaves. Thus, PCD initiation in *lsd1* is dependent on environmental triggers, particularly light. Photosynthetic electron transport inhibitors block lesion formation in *lsd1*. Lesion development was favored by high light and low CO_2, implicating photorespiratory

H_2O_2 production in the PCD response (Mateo et al., 2004). Moreover, a major leaf isoform of catalase (CAT1) is decreased when PCD is initiated in *lsd1* (Mateo et al., 2004).

Interestingly, PCD is also observed in plants deficient in the major catalase isoforms (Rizhsky et al., 2002). Because very high rates of H_2O_2 production occur during photosynthesis, particularly in the peroxisomes and chloroplasts (Noctor et al., 2002), we conclude that ROS-triggered PCD can occur in two ways. Either ROS production can be activated in compartments where antioxidant buffering is low (e.g., the apoplast) or antioxidant capacity can be withdrawn from compartments in which ROS production is high (e.g., the chloroplast and peroxisome). The extent of interplay between these individual processes and the relationship of each to reactive nitrogen species accumulation remains to be established. However, the fact that several key antioxidative enzymes are dual targeted to chloroplasts and mitochondria (Chew et al., 2003) may be significant in PCD regulation.

Much attention has focused on the role of salicylic acid in the regulation of PCD and the induction of PR proteins associated with systemic acquired resistance. Both ROS and antioxidants have been strongly implicated in salicylic acid signaling. Glutathione is able to induce PR transcript induction (Gomez et al., 2004 and references cited therein), whereas localized cell death occurs in ascorbate-deficient plants (Pastori et al., 2003). These effects point to opposing functions for ascorbate and glutathione in redox signal transduction and are likely related to redox modulation of NPR1, a protein necessary for salicylic acid signaling. It has been shown that NPR1 exists as an oligomer formed by intermolecular disulfide bonds and that induction of systemic acquired resistance leads to a change in cellular reduction potential that reduces NPR1 to its monomeric form, which accumulates in the nucleus and activates target gene expression (Mou et al., 2003). Furthermore, the interaction of NPR1 with the transcription factor TGA1 and resulting induction of TGA1 DNA binding activity is dependent on the reduction of TGA1 Cys residues induced by SA (Després et al., 2003). In addition, ascorbate deficiency is associated with upregulation of extracellular defenses (nonspecific peroxidases) and constitutive activation of processes known to be controlled by apoplastic ROS production, notably abscisic acid signaling, pathogen resistance, and constitutive expression of PR proteins (Pastori et al., 2003; Barth et al., 2004). In the ascorbate-deficient *vtc1* mutant, these effects are accompanied by enhanced glutathione and nuclear localization of NPR1 (our unpublished data).

Based on these and other recent data, the hypothetical scheme shown in Figure 3 illustrates some of the likely roles of cellular reductants and antioxidants in ROS signaling linked to PCD and salicylic acid signaling. Key points are, first, that redox signaling during an incompatible plant–pathogen interaction involves both oxidative and reductive steps and, second, that there is a relationship between salicylic acid, catalase (and probably also ascorbate peroxidase), and glutathione in this process (Vanacker et al., 2000; Mou et al., 2003). Reductive signaling triggered by an upstream oxidative signal parallels ROS-mediated signaling in mammalian cells through the transcription factors NF-κB and AP-1. Binding of these transcription factors to DNA is favored by a reducing environment and is inhibited by GSSG or oxidized thioredoxin (Dröge, 2002). The extent to which the processes shown in Figure 3 occur in the same cells is unknown, but as noted above, ROS signaling can be initiated first in chloroplasts after exposure to ozone, and this contributes to the activation of membrane-associated NADPH oxidases that are involved in intercellular redox signal transduction (Joo et al., 2005). Redox signals can then be transmitted via H_2O_2 locally in the apoplast or long distance through the vascular system where this oxidant has a longer lifetime than in antioxidant-rich compartments or tissues.

Recent evidence strongly suggests that cell death triggered by either singlet oxygen (Wagner et al., 2004) or paraquat (Chen and Dickman, 2004) in the chloroplast does not result from oxidative physiochemical damage but is the consequence of activation of a genetic program. In animals, pro- and anti-apoptotic factors control mitochondria-dependent cell death pathways. Early work in this area showed that expression of a human anti-apoptotic factor in tobacco did not confer protection against pathogen-induced cell death (Mittler et al., 1996). More recently, however, several studies have shown that animal PCD regulators modulate plant PCD and stress responses. Expression of the mammalian pro-apoptotic protein Bax promoted death, whereas the anti-apoptotic protein Bcl2 suppressed PCD in rice cells challenged with elicitors (Matsumura et al., 2003). Interestingly, overexpression of glutathione peroxidase inhibited Bax- and oxidative stress–induced PCD in tomato (Chen et al., 2004). Bleaching of tobacco leaves induced by superoxide and singlet oxygen-generating herbicides was prevented by expression of either mammalian Bcl proteins or *Caenorhabditis elegans* CED-9 (Chen and Dickman, 2004). These results confirm that plant PCD can be regulated by pro- and anti-apoptotic factors that interact with ROS. Moreover, they suggest that the mechanisms through which ROS-generating chloroplast herbicides kill plants may involve PCD rather than physicochemical damage that overwhelms the antioxidative system.

CONCLUSIONS AND PERSPECTIVES

Redox signal transduction is a universal feature of aerobic life honed through evolution to balance information from metabolism and the environment. Here, we have presented a view of the plant cell as a series of interconnecting compartments with different antioxidant buffering capacities determined by differences in synthesis, transport, and/or degradation. The result is a set of discrete locations where signaling is controlled (or buffered) independently. This permits redox-sensitive signal transduction to occur in locations such as the apoplast, the thylakoid, and, perhaps, the endoplasmic reticulum, whereas other highly buffered spaces have a much higher threshold for ROS signals. Both oxidants

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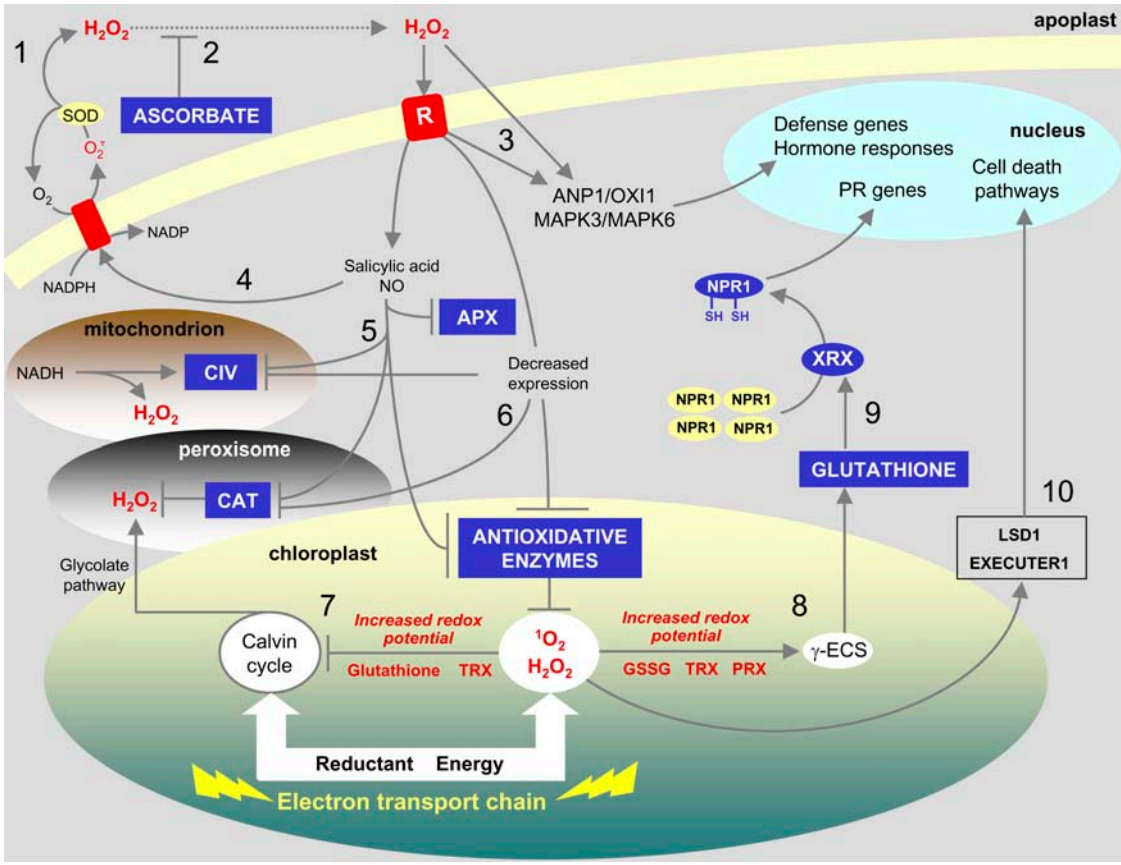

Figure 3. Oxidant and Antioxidant Signaling in Cell Death and Acclimation Responses: Major Components of the Metabolic Interface between Plant Stresses and Orchestrated Responses.

The hypothetical scheme draws together some of the redox-modulated elements recently identified in plants and others likely to be influential. Components that promote an oxidative signal are shown in red, and those that oppose such signals or transmit a reductive signal are shown in dark blue. Plasma membrane NADPH oxidases are activated by elicitor or hormone-mediated signaling and produce H_2O_2 (1). Production of H_2O_2 can oxidize putative membrane receptors, and this function is opposed by apoplastic ascorbate (2). ROS production activates signaling through specific MAP kinases (3), and some of these components are also important in ROS-mediated hormone and growth responses. A key factor in specification of pathogenesis and cell death responses is secondary production of ROS, either by positive feedback enhancement of the primary oxidative signal (4) or by withdrawal and inactivation of antioxidative capacity. Downregulation of the antioxidative system could occur by posttranslational modulation of heme functions by salicylic acid and nitric oxide (5) and/or programmed withdrawal of antioxidative enzyme expression (6). Oxidative inhibition of chloroplast metabolism by glutathionylation and/or inactivation of thioredoxin-modulated enzymes may also be crucial in increasing chloroplastic flux to oxygen to enhance ROS production (7). Greater availability of ROS activates glutathione synthesis (8), and the resulting increase in cytosolic glutathione is somehow linked to induction or activation of a thioredoxin or glutaredoxin that is able to reduce NPR1 (9). NPR1 reduction is associated with its accumulation in the nucleus and its interaction with TGA transcription factors to induce *PR* gene expression. Enhanced chloroplastic levels of ROS such as singlet oxygen may also activate the pathways that (10) set in train the cell death program. For discussion and references, see text. APX, ascorbate peroxidase; CAT, catalase; CIV, cytochrome oxidase; SOD, superoxide dismutase.

and antioxidants fulfill signaling roles to provide information on general plant health, particularly in terms of robustness for defense, using kinase-dependent and independent pathways that are initiated by redox-sensitive receptors modulated by thiol status. Antioxidants are not passive bystanders in this crosstalk, but rather function as key signaling compounds that constitute a dynamic metabolic interface between plant cell stress perception and physiological responses. Current data suggest that glutathione is a key arbiter of the intracellular redox potential, and ascorbate is particularly influential in setting thresholds for apoplastic and cytoplasmic signaling. Differential antioxidant concentrations between compartments permit antioxidant-driven vectorial signaling through processes such as ascorbate-driven electron transport or futile cycles. The future will

determine more precisely how ascorbate, glutathione, and tocopherol are involved in initiating and controlling redox signal transduction and how they trigger other related responses such as PR gene expression to optimize survival strategies.

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REFERENCES

Ahn, S.-G., and Thiele, D.J. (2003). Redox regulation of mammalian heat shock factor 1 is essential for Hsp gene activation and protection from stress. Genes Dev. **17,** 516–528.

Ameisen, J.C. (2002). On the origin, evolution, and nature of programmed cell death: A timeline of four billion years. Cell Death Differ. **9**, 367–393.

Asada, K., and Takahashi, M. (1987). Production and scavenging of active oxygen species in photosynthesis. In Photoinhibition, D. Kyle, C. Osmond, and C. Arntzen, eds (New York: Elsevier Science Publishers), pp. 227–287.

Ball, L., Accotto, G., Bechtold, U., Creissen, G., Funck, D., Jimenez, A., Kular, B., Leyland, N., Mejia-Carranza, J., Reynolds, H., Karpinski, S., and Mullineaux, P.M. (2004). Evidence for a direct link between glutathione biosynthesis and stress defense gene expression in Arabidopsis. Plant Cell **16,** 2448–2462.

Balmer, Y., Vensel, W.H., Tanaka, C.K., Hurkman, W.J., Gelhaye, E., Rouhier, N., Jacquot, J.P., Manieri, W., Schürmann, P., Droux, M., and Buchanan, B. (2004). Thioredoxin links redox to the regulation of fundamental processes of plant mitochondria. Proc. Natl. Acad. Sci. USA **101,** 2642–2647.

Barth, C., Moeder, W., Klessig, D.F., and Conklin, P.L. (2004). The timing of senescence and response to pathogens is altered in the ascorbate-deficient mutant vitamin C-1. Plant Physiol. **134,** 1784–1792.

Bauer, C.E., Elsen, S., and Bird, T.H. (1999). Mechanisms for redox control of gene expression. Annu. Rev. Microbiol. **53,** 495–523.

Bors, W., Heller, W., Michel, C., and Saran, M. (1990). Flavonoids as antioxidants: Determination of radical-scavenging efficiencies. In Oxygen Radicals in Biological Systems: Oxygen Radicals and Antioxidants, Methods in Enzymology, Vol. 186, L. Packer and A.N. Glazer, eds (New York: Elsevier Science Publishers), pp. 343–355.

Bors, W., Langebartels, C., Michel, C., and Sandermann, H. (1989). Polyamines as radical scavengers and protectants against ozone damage. Phytochemistry **28,** 1589–1595.

Chen, S., and Dickman, M.B. (2004). Bcl-2 family members localize to tobacco chloroplasts and inhibit programmed cell death induced by chloroplast-targeted herbicides. J. Exp. Bot. **55,** 2617–2623.

Chen, S., Vaghchipawala, Z., Li, W., Asard, H., and Dickman, M.B. (2004). Tomato phospholipid hydroperoxide glutathione peroxidase inhibits cell death induced by bax and oxidative stress in yeast and plants. Plant Physiol. **135,** 1630–1641.

Chew, O., Whelan, J., and Millar, A.H. (2003). Molecular definition of the ascorbate-glutathione cycle in Arabidopsis mitochondria reveals dual targeting of antioxidant defenses in plants. J. Biol. Chem. **278,** 46869–46877.

Collin, V., Lamkemeyer, P., Miginiac-Maslow, M., Hirasawa, M., Knaff, D.B., Dietz, K.J., and Issakidis-Bourguet, E. (2004). Characterization of plastidial thioredoxins from Arabidopsis belonging to the new y-type. Plant Physiol. **136,** 4088–4095.

Creissen, G., Firmin, J., Fryer, M., Kular, B., Leyland, M., Reynolds, H., Pastori, G., Wellburn, F., Baker, N.R., Wellburn, A., and Mullineaux, P. (1999). Elevated glutathione biosynthetic capacity in the chloroplasts of transgenic tobacco paradoxically causes increased oxidative stress. Plant Cell **11,** 1277–1291.

Cross, J.V., and Templeton, D.J. (2004). Oxidative stress inhibits MEKK1 by site-specific glutathionylation in the ATP-binding domain. Biochem. J. **381,** 675–683.

Dangl, J.L., and Jones, J.D.G. (2001). Plant pathogens and integrated defence responses to infection. Nature **411,** 826–833.

Davletova, S., Rizhsky, L., Liang, H., Shengqiang, Z., Oliver, D.J., Coutu, J., Shulaev, V., Schlauch, K., and Mittler, R. (2005). Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of Arabidopsis. Plant Cell **17,** 268–281.

Delauney, A., Pflieger, D., Barrault, M.B., Vinh, J., and Toledano, M.B. (2002). A thiol peroxidase is an H_2O_2 receptor and redox-transducer in gene activation. Cell **111,** 1–11.

De Pinto, M.C., and De Gara, L. (2004). Changes in the ascorbate metabolism of apoplastic and symplastic spaces are associated with cell differentiation. J. Exp. Bot. **55,** 2559–2569.

Desikan, R., Hancock, J.T., Bright, J., Harrison, J., Weir, I., Hooley, R., and Neill, S.J. (2005). A role for ETR1 in hydrogen peroxide signaling in stomatal guard cells. Plant Physiol. **137,** 831–834.

Després, C., Chubak, C., Rochon, A., Clark, R., Bethune, T., Desveaux, D., and Fobert, P.R. (2003). The Arabidopsis NPR1 disease resistance protein is a novel cofactor that confers redox regulation of DNA binding activity to the basic domain/leucine zipper transcription factor TGA1. Plant Cell **15,** 2181–2191.

Dröge, W. (2002). Free radicals in the physiological control of cell function. Physiol. Rev. **82,** 47–95.

Dutilleul, C., Garmier, M., Noctor, G., Mathieu, C.D., Chétrit, P., Foyer, C.H., and De Paepe, R. (2003). Leaf mitochondria modulate whole cell redox homeostasis, set antioxidant capacity and determine stress resistance through altered signaling and diurnal regulation. Plant Cell **15,** 1212–1226.

Evans, N.H., McAinsh, M.R., Hetherington, A.M., and Knight, M.R. (2005). ROS perception in *Arabidopsis thaliana*: The ozone-induced calcium response. Plant J. **41,** 615–626.

Foreman, J., Demidchik, V., Bothwell, J.H., Mylona, P., Miedema, H., Torres, M.A., Linstead, P., Costa, S., Brownlee, C., Jones, J.D., Davies, J.M., and Dolan, L. (2003). Reactive oxygen species produced by NADPH oxidase regulate plant cell growth. Nature **27,** 442–446.

Foyer, C.H., and Lelandais, M. (1996). A comparison of the relative rates of transport of ascorbate and glucose across the thylakoid, chloroplast and plasma membranes of pea leaf mesophyll cells. J. Plant Physiol. **148,** 391–398.

Foyer, C.H., Trebst, A., and Noctor, G. (2005). Protective and signalling functions of ascorbate, glutathione and tocopherol in chloroplasts. In Advances in Photosynthesis and Respiration: Photoprotection, Photoinhibition, Gene Regulation, and Environment, Vol. 19, B. Demmig-Adams and W.W. Adams, eds

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(Dordrecht, The Netherlands: Springer Science Publishers), pp. 241–268.

Gomez, L.D., Noctor, G., Knight, M., and Foyer, C.H. (2004). Regulation of calcium signaling and gene expression by glutathione. J. Exp. Bot. **55,** 1851–1859.

Green, M.A., and Fry, S.C. (2005). Vitamin C degradation in plant cells via enzymatic hydrolysis of 4-*O*-oxalyl-L-threonate. Nature **433,** 83–87.

Gupta, R., and Luan, S. (2003). Redox control of protein tyrosine phosphatases and mitogen-activated protein kinases in plants. Plant Physiol. **132,** 1149–1152.

Horemans, N., Foyer, C.H., and Asard, H. (2000). Transport and action of ascorbate at the plant plasma membrane. Trends Plant Sci. **5,** 263–267.

Ito, H., Iwabuchi, M., and Ogawa, K. (2003). The sugar-metabolic enzymes aldolase and triose-phosphate isomerase are targets of glutathionylation in *Arabidopsis thaliana*: Detection using biotinylated glutathione. Plant Cell Physiol. **44,** 655–660.

Jabs, T., Dietrich, R.A., and Dangl, J.L. (1996). Initiation of runaway cell death in an *Arabidopsis* mutant by extracellular superoxide. Science **273,** 1853–1856.

Joo, J.H., Wang, S., Chen, J.G., Jones, A.M., and Fedoroff, N.V. (2005). Different signaling and cell death roles of heterotrimeric G protein a and b subunits in the Arabidopsis oxidative stress response to ozone. Plant Cell **17,** 957–970.

Kanwischer, M., Porfirova, S., Bergmuller, E., and Dormann, P. (2005). Alterations in tocopherol cyclase activity in transgenic and mutant plants of Arabidopsis affect tocopherol content, tocopherol composition and oxidative stress. Plant Physiol. **137,** 713–723.

König, J., Baier, M., Horling, F., Kahmann, U., Harris, G., Schurmann, P., and Dietz, K.J. (2002). The plant-specific function of 2-Cys peroxiredoxin-mediated detoxification of peroxides in the redox-hierarchy of photosynthetic electron flux. Proc. Natl. Acad. Sci. USA **99,** 5738–5743.

Kovtun, Y., Chiu, W.-L., Tena, G., and Sheen, J. (2000). Functional analysis of oxidative stress-activated mitogen-activated protein kinase cascade in plants. Proc. Natl. Acad. Sci. USA **97,** 2940–2945.

Kranner, I., Cram, W.J., Zorn, M., Wornik, S., Yoshimura, I., Stabentheiner, E., and Pfeifhofer, W. (2005). Antioxidants and photoprotection in a lichen as compared with its isolated symbiotic partners. Proc. Natl. Acad. Sci. USA **102,** 3141–3146.

Kwak, J.M., Mori, I.C., Pei, Z.M., Leonhardt, N., Torres, M.A., Dangl, J.L., Bloom, R.E., Bodde, S., Jones, J.D., and Schroeder, J.I. (2003). NADPH oxidase *AtrbohD* and *AtrbohF* genes function in ROS-dependent ABA signaling in Arabidopsis. EMBO J. **22,** 2623–2633.

Lecourieux, D., Mazars, C., Pauly, N., Ranjeva, R., and Pugin, A. (2002). Analysis and effects of cytosolic free calcium increases in response to elicitors in *Nicotiana plumbaginifolia* cells. Plant Cell **14,** 2627–2641.

Lemaire, S.D., Guillon, B., Le Maréchal, P., Keryer, E., Miginiac-Maslow, M., and Decottignies, P. (2004). New thioredoxin targets in the unicellular photosynthetic eukaryote *Chlamydomonas reinhardtii.* Proc. Natl. Acad. Sci. USA **101,** 7475–7480.

Mateo, A., Mühlenbock, P., Rustérucci, C., Chang, C.C.C., Miszalski, Z., Karpinska, B., Parker, J.E., Mullineaux, P.M., and Karpinski, S. (2004). *LESION SIMULATING DISEASE 1* is required for acclimation to conditions that promote excess excitation energy. Plant Physiol. **136,** 2818–2830.

Matsumura, H., Nirasawa, S., Kiba, A., Urasaki, N., Saitoh, H., Ito, M., Kawai-Yamada, M., Uchimiya, H., and Terauchi, R. (2003). Overexpression of Bax inhibitor suppresses the fungal elicitor-induced cell death in rice (*Oryza sativa* L.) cells. Plant J. **33,** 425–437.

Maxwell, D.P., Nickels, R., and McIntosh, L. (2002). Evidence of mitochondrial involvement in the transduction of signals required for the induction of genes associated with pathogen attack and senescence. Plant J. **29,** 269–279.

Mittler, R., Shulaev, V., Seskar, M., and Lam, E. (1996). Inhibition of programmed cell death in tobacco plants during a pathogen-induced hypersensitive response at low oxygen pressure. Plant Cell **8,** 1991–2001.

Mou, Z., Fan, W., and Dong, X. (2003). Inducers of plant systemic acquired resistance regulate NPR1 function through redox changes. Cell **113,** 935–944.

Mur, L.A., Kenton, P., and Draper, J. (2005). *In planta* measurements of oxidative bursts elicted by avirulent and virulent bacterial pathogens suggests that H_2O_2 is insufficient to elicit cell death in tobacco. Plant Cell Environ. **28,** 548–561.

Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L., and Foyer, C.H. (2002). Drought and oxidative load in wheat leaves: A predominant role for photorespiration? Ann. Bot. (Lond.) **89,** 841–850.

Pastori, G.M., Kiddle, G., Antoniw, J., Bernard, S., Veljovic-Jovanovic, S., Verrier, P.J., Noctor, G., and Foyer, C.H. (2003). Leaf vitamin C contents modulate plant defense transcripts and regulate genes that control development through hormone signaling. Plant Cell **15,** 939–951.

Pignocchi, C., Fletcher, J.E., Barnes, J., and Foyer, C.H. (2003). The function of ascorbate oxidase (AO) in tobacco (*Nicotiana tabacum* L.). Plant Physiol. **132,** 1631–1641.

Polle, A. (2001). Dissecting the superoxide dismutase-ascorbate-glutathione pathway in chloroplasts by metabolic modeling. Computer simulations as a step towards flux analysis. Plant Physiol. **126,** 445–462.

Potters, G., Horemans, N., Bellone, S., Caubergs, J., Trost, P., Guisez, Y., and Asard, H. (2004). Dehydroascorbate influences the plant cell cycle through a glutathione-independent reduction mechanism. Plant Physiol. **134,** 1479–1487.

Preger, V., Scagliarini, S., Pupillo, P., and Trost, P. (2004). Identification of an ascorbate-dependent cytochrome *b* of the tonoplast membrane sharing biochemical features with members of the cytochrome b561 family. Planta **220,** 365–375.

Rentel, M.C., Lecourieux, D., Ouaked, F., Usher, S.L., Peterson, L., Okamoto, H., Knight, H., Peck, S.C., Grierson, C.S., Hirt, H., and Knight, M.R. (2004). OXI1 kinase is necessary for oxidative burst-mediated signalling in Arabidopsis. Nature **427,** 858–861.

Rey, P., Cuiné, S., Eymery, F., Garin, J., Court, M., Jacquot, J.P., Rouhier, N., and Broin, M. (2005). Analysis of the proteins targeted by CDSP32, a plastidic thioredoxin participating in oxidative stress responses. Plant J. **41,** 31–42.

Rizhsky, L., Hallak-Herr, E., Van Breusegem, F., Rachmilevitch, S., Barr, J.E., Rodermel, S., Inzé, D., and Mittler, R. (2002). Double antisense plants lacking ascorbate peroxidase and catalase are less sensitive to oxidative stress than single antisense plants lacking ascorbate peroxidase or catalase. Plant J. **32,** 329–342.

Rouhier, N., Gelhaye, E., Sautiere, P.E., Brun, A., Laurent, P., Tagu, D., Gerard, J., De Fay, E., Meyer, Y., and Jacquot, J.P. (2001). Isolation and characterization of a new peroxiredoxin from poplar sieve tubes that uses either glutaredoxin or thioredoxin as a proton donor. Plant Physiol. **127,** 1299–1309.

Setterdahl, A.T., Chivers, P.T., Hirasawa, M., Lemaire, S.D., Keryer, E., Miginiac-Maslow, M., Kim, S.K., Mason, J., Jacquot, J.P., Longbine, C.C., de Lamotte-Guery, F., and Knaff, D.B. (2003). Effect of pH on the

oxidation-reduction properties of thioredoxins. Biochemistry **42,** 14877–14884.

Tokunaga, T., Miyahara, K., Tabata, K., and Esaka, M. (2005). Generation and properties of ascorbic acid-overproducing transgenic tobacco cells expressing sense RNA for L-galactono-1,4-lactone dehydrogenase. Planta **220,** 854–863.

Vanacker, H., Carver, T.L.W., and Foyer, C.H. (2000). Early H_2O_2 accumulation in mesophyll cells leads to induction of glutathione during the hypersensitive response in the barley-powdery mildew interaction. Plant Physiol. **123,** 1289–1300.

Vernoux, T., Wilson, R.C., Seeley, K.A., Reichheld, J.P., Muroy, S., Brown, S., Maughan, S.C., Cobbett, C.S., Van Montagu, M., Inzé, D., May, M.J., and Sung, Z.R. (2000). The ROOT MERISTEMLESS1/CADMIUM SENSITIVE2 gene defines a glutathione-dependent pathway involved in initiation and maintenance of cell division during postembryonic root development. Plant Cell **12,** 97–110.

Wachter, A., Wolf, S., Steiniger, H., Bogs, J., and Rausch, T. (2005). Differential targeting of *GSH1* and *GSH2* is achieved by multiple transcription initiation: Implications for the compartmentation of glutathione biosynthesis in the *Brassicaceae*. Plant J. **41,** 15–30.

Wagner, D., Przybyla, D., Op den Camp, R., Kim, C., Landgraf, F., Lee, K.P., Würsch, M., Laloi, C., Nater, M., Hideg, E., and Apel, K. (2004). The genetic basis of singlet oxygen-induced stress responses of *Arabidopsis thaliana*. Science **306,** 1183–1185.

Yamazaki, D., Motohashi, K., Kasama, T., Hra, Y., and Hisabori, T. (2004). Target proteins of the cytosolic thioredoxins in *Arabidopsis thaliana*. Plant Cell Physiol. **45,** 18–27.